Supplementary Methods

Flow Cytometry:

Single cell suspensions from all samples were stained with Live Dead Blue (Invitrogen, 1:400 in PBS) for 10 minutes at 4°C, washed with FACs Buffer (1% FBS in PBS) and then stained with cell surface marker antibodies for 45 minutes, 4°C. For Ki-67 and FoxP3 staining, cells were fixed with eBioscience Fix/Perm for one hour at 4°C, and washed two times with eBioscience Perm/Wash Buffer. Cells were stained in presence of Perm Wash for 45 minutes at 4°C.

Antibodies used were anti-human CD3 (UCHT1, SK7), CD4 (OKT4), CD69 (FN50), CD103 (Ber-Act8), PD-1 (EH12-H7), TIM3 (F38-2E2), CD62L (DREG-56), CD45RO (UCHL1), CD39 (A1), Foxp3 (206D), CD45RA (HI100), PDL-1 (M1H1), CD14 (M5E2), CD15 (HI98) from Biolegend; Anti-human CD8 (RPA-T8), CTLA-4 (BN13), from BD Bioscience; and anti-human TIGIT (MBAS43), Eomes (WD1928) and Ki-67 (20Raj1) from eBiosciences.

Intracellular cytokine staining was performed as detailed previously (16). Cytokine antibodies used were directly conjugated anti-human IFN–γ (4S.B3), TNF-α (MAb11), and IL-2 (MQ1-17H12) from Biolegend. For concurrent transcription factor and cytokine staining, the cells were treated by the eBioscience FoxP3 staining protocol. Flow Cytometric analysis was performed on a BD Fortessa (BD Pharmingen).

Labeled cells were washed and re-suspended in FACs buffer for flow cytometric analysis on a BD Fortessa with FACS Diva Software (BD Biosciences) and analyzed using FlowJo 10.2. Negative gating was based on a “fluorescence minus one” (FMO)

strategy. viSNE, a visualization tool of high-dimensional single cell data based on t-Distributed Stochastic Neighbor Embedding (t-SNE) algorithm (29) was generated via FCS Express 6 (De Novo Software).

Bispecific T cell engager (BiTE) killing assay: To measure cytotoxicity, we employed a BiTE killing assay similar to that recently reported (4). A lentiviral expression vector (17) (graciously provided by Dr. Yang Bing Zhao, UPenn) was constructed to produce a BiTE by fusing the scFV from blinitumamab (that recognizes CD3) with the scFV from SS1 that recognizes mesothelin. 293 cells were transduced by the vector and supernatant was collected. We also generated a human mesothelioma cell line that expressed high levels of mesothelin and luciferase (EMMESO) (18). The killing assay was performed by plating EMMESO target tumor cells at 5000/well in a 96 well plate, and allowing them to adhere over a 4-6 hour period. A volume of digested patient tumor cell suspension was then added to the EMMESO cells based on live CD8+ T cells frequency (as measured by flow cytometry) to achieve a ratio of ~10 CD8+ T cells to 1 EMMESO tumor cell. 30 μl of BiTE supernatant or control media was added to some wells of those cocultures. After 18 hours of coculture, the supernatant from the wells was aspirated, the remaining tumor cells were washed and then lysed. Luminescence from the remaining tumor cells was measured using a Glomax Luminometer (Promega, Inc). Percent lysis was measured using the formula % killing = 100 – [(luminescence of tumor alone well – luminescence of cocultured well) / (luminescence of tumor alone well) x 100]. BiTE-induced killing was the difference in % lysis measured in BiTE + digest + tumor wells and digest + tumor wells. As a control, we used the EMParental cell line (a mesothelin-negative mesothelioma cell line).

Heat Map Data: Data (non-log transformed) was visualized in a heat map format using the Morpheus software (https://software.broadinstitute.org/morpheus/).